

Articles

Synthesis of 1-Boraadamantaneamine Derivatives with Selective Astrocyte vs C6 Glioma Antiproliferative Activity. A Novel Class of Anti-Hepatitis C Agents with Potential to Bind CD81

Carl E. Wagner,[†] Michael L. Mohler,[§] Gyong Suk Kang,[§] Duane D. Miller,[§] Eldon E. Geisert,[#] Yu-An Chang,[†] Everly B. Fleischer,[‡] and Kenneth J. Shea^{*,*}

Department of Chemistry, University of California—Irvine, Irvine, California 92697, College of Pharmacy and College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee 38163, and RIC Biotech & Trading, Inc., Orange County, California 92614

Received July 24, 2002

A variety of amine complexes with 1-boraadamantane were synthesized and subsequently evaluated for an antiproliferative effect on CD81-enriched cell lines to provide evidence for binding and activation of CD81. CD81 is a member of the tetraspanin family of membrane proteins found in all cell lineages in the liver. CD81 signals for antiproliferation when bound by antibodies. It is known that the HCV-E2 envelope glycoprotein binds to the CD81 protein. While it is unclear whether virus entry into host cells is directly linked to virus attachment via CD81 for HCV, this step in the viral life cycle has recently proven to be an effective point of attack for other viruses including HIV and rhinoviruses. The aim of the current study concerns the synthesis of amantidine analogues by appending primary amines to 1-boraadamantane to evaluate such compounds for CD81-dependent antiproliferation of CD81-enriched cell lines (astrocyte) vs CD81-deficient cell lines (C6 glioma). If the antiproliferative effect of these amantidine analogues proves to be an effect of binding and activating CD81, then these compounds may have the potential to prevent or treat HCV infections. Each compound's potential for preventive and therapeutic activity stems from the compound's potential to block viral attachment, virus–cell fusion, or virus entry into host cells or to counter potential mechanisms of HCV immune evasion. Out of a library of over 500 compounds, including randomly selected small molecules and rationally designed small molecules, only the 1-boraadamantaneamine compounds and structurally similar analogues display a significant antiproliferative effect on the CD81-enriched astrocytes relative to the CD81-deficient cell lines. In fact, 1-boraadamantane·L-phenylalanine methyl ester complex (**5**), 1-boraadamantane·ethanolamine complex (**8**), and (*S*)-2-[(adamantane-1-carbonyl)amino]-3-phenylpropionic acid (**15**) show a dose-dependent, astrocyte-selective antiproliferative activity in the concentration range 0.1–10 μ M. This is consistent with the binding and activation of CD81 and represents a 2-fold improvement compared to the clinically prescribed anti-HCV agent, amantidine, in the same concentration range. Consequently, the 1-boraadamantaneamine derivatives present a promising lead in the development of small molecules with potential to bind to CD81 and treat HCV infections.

Introduction

The hepatitis C virus (HCV) affects a substantial part of the world's population, from 1% to 2%, and complications arising from the progression of the chronic infection of the liver include cirrhosis and liver cancer.¹ Currently, treatment of HCV with α -interferons and ribavirin affects a continued remittance of viral RNA in less than 50% of treated patients.² Recent studies for

the treatment of hepatitis C with amantidine alone and in combination with α -interferon and ribavirin have shown promise in the reduction of alanine aminotransferase (ALT), the enzyme released by the lysis of damaged liver cells, and HCV RNA levels.³ Other methods of treatment currently under investigation involve viral protease inhibitors, but those developed thus far include only a small variety of peptide substrates and analogues.⁴ With the difficulty of administering small peptides as therapeutic drugs and the promise shown by drugs such as amantidine, there is ample motivation to synthesize small-molecule analogues of 1-aminoadamantane to be tested for activity against hepatitis C.

* To whom correspondence should be addressed. Phone: (949) 824-5844. Fax: (949) 824-2210. E-mail: kjshea@uci.edu.

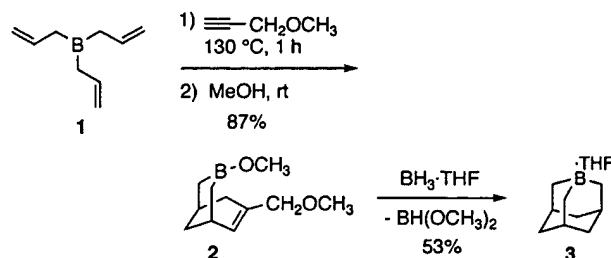
[†] University of California—Irvine.

[§] College of Pharmacy, University of Tennessee Health Science Center.

[#] College of Medicine, University of Tennessee Health Science Center.

[‡] RIC Biotech & Trading, Inc.

Scheme 1



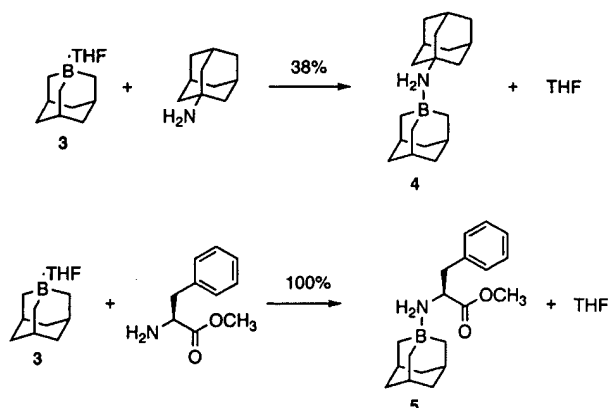
While the mechanism by which small molecules such as amantidine effect a remittance of ALT and HCV RNA levels is not well understood, it may involve blocking viral adhesion to liver cells and subsequent infection. Recently, the large extracellular loop of the CD81 transmembrane protein (CD81-LEL), a protein common to all cell lineages in the liver, has been shown to bind to the HCV-E2 envelope glycoprotein.⁵ The CD81-LEL has been crystallized, and its crystal structure has been reported at 1.6 Å resolution.⁶ In fact, the HCV-E2 binds to CD81-LEL with a K_d of 1.8 nM at 25 °C,⁷ and the region in the CD81-LEL that participates in E2 binding is composed of largely hydrophobic amino acid residues. While it is not known whether the CD81 protein serves as a receptor for HCV entry into the cell, it arguably serves as a receptor for virus attachment. Indeed, the pronounced anti-HCV activity of 1-aminoadamantane hydrochloride could possibly be attributed to a binding interaction with the CD81-LEL. Molecular modeling of this region may help to elucidate the key binding interactions of molecules incorporating the 1-aminoadamantane core motif and aid in the development of new small-molecule drugs to bind to CD81 and treat hepatitis C.

Owing to the versatility with which functionalized primary amines can be appended to the 1-boraadamantane core, as well as the stability of the resulting complexes, 1-boraadamantane represents a promising substrate for modification and pharmacology studies en route to 1-aminoadamantane analogues. In fact, several 1-boraadamantane complexes with amines and other nitrogen-containing compounds display a pronounced antiviral effect against the influenza A and B viruses.⁸ On the basis of the evidence for the potent antiviral pharmacology of 1-boraadamantaneamine complexes and the structural similarities to 1-aminoadamantane, a number of 1-boraadamantaneamine complexes were synthesized to be evaluated for a CD81-dependent antiproliferative activity in astrocytes vs C6 glioma.

Chemistry

The 1-boraadamantaneamine complexes synthesized for testing were conveniently derived from 1-boraadamantane·THF (3) in high yield by stoichiometric addition. Thus, 1-boraadamantane·THF (3) was synthesized by the hydroboration of 3-methoxy-7-(methoxymethyl)-3-borabicyclo[3.3.1]non-6-ene (2). This compound was prepared by the reaction of triallylborane (1) with methyl propargyl ether followed by methanolysis, according to the procedure of Mikhailov and co-workers (Scheme 1).⁹ Triallylborane (1) was synthesized accord-

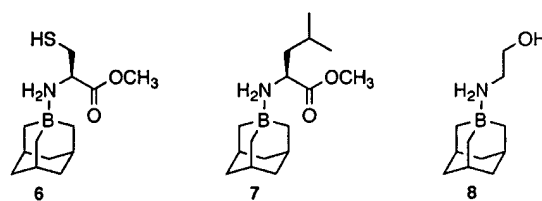
Scheme 2



ing to the method of Zakharkin and co-workers.¹⁰ After purification by sublimation, 1-boraadamantane·THF was procured in 46% overall yield from triallylborane.

The first 1-boraadamantaneamine complexes to be synthesized and tested included complexes with 1-aminoadamantane (4) and L-phenylalanine methyl ester (5) (Scheme 2). Complex 4 was assembled in 38% yield by addition of a stoichiometric amount of 1-aminoadamantane to 1-boraadamantane·THF and purification by recrystallization in ethanol, according to the protocol of Mikhailov and co-workers.^{9a} Complex 5 was synthesized in quantitative yield by stoichiometric addition of L-phenylalanine methyl ester to 1-boraadamantane·THF.

By use of the same protocol to synthesize amine complex 5 (Scheme 2), complexes of 1-boraadamantane with L-cysteine methyl ester (6) and L-leucine methyl ester (7) were synthesized in quantitative yield. Additionally, a complex of 1-boraadamantane with ethanolamine (8) was also synthesized in quantitative yield for testing. The amine complexes 6–8 are air-stable,



crystalline complexes. X-ray diffraction was performed on single crystals of complexes 5, 6, and 8 (Figure 1).

The X-ray diffraction study for complex 5 reveals that the amine group of L-phenylalanine methyl ester coordinates to boron in boraadamantane. The crystal structure of 6 establishes that the amine group coordinates to boron rather than the thiol group of L-cysteine methyl ester, and the X-ray crystal structure of 8 reveals that the amine group coordinates to boron rather than the hydroxyl group of ethanolamine. The polarity of the L-cysteine methyl ester as well as the free thiol group improves the solubility of the amino acid methyl ester complex 6 in polar, protic solvents. The compact size of ethanolamine and its free hydroxyl group also improves the solubility of complex 8 in polar, protic solvents. In fact, the solubility of complexes 4–8 in water ranged from 3.8 mg/mL for complex 8 to 0.6 mg/mL for complex 5. With the rare exceptions of triallylboranes, the

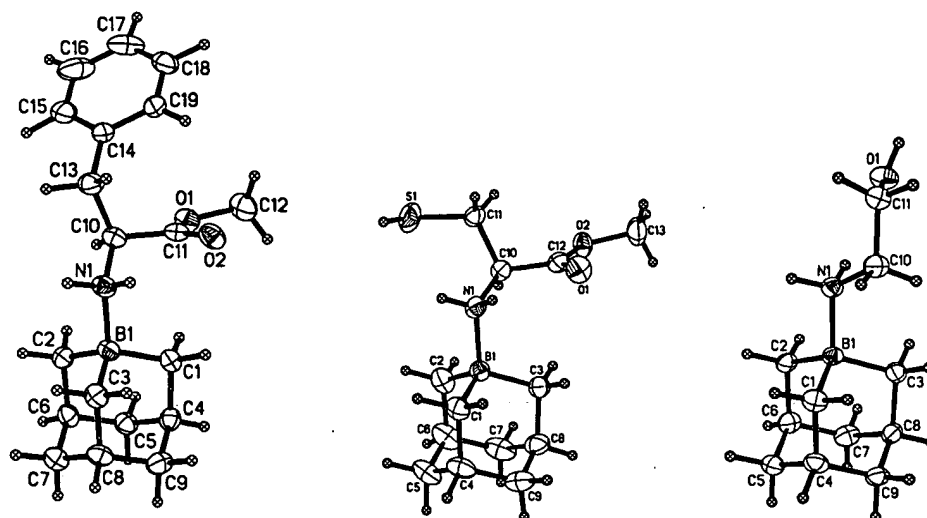
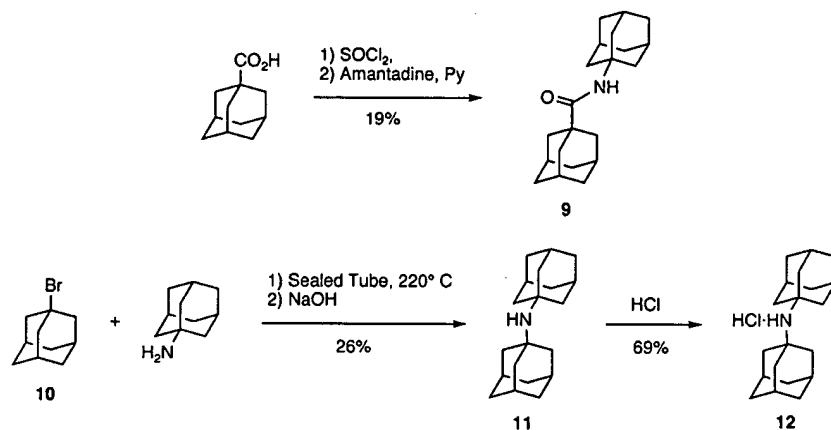


Figure 1. X-ray crystal structures of 1-boraadamantane·L-phenylalanine methyl ester (**5**), 1-boraadamantane·L-cysteine methyl ester (**6**), and 1-boraadamantane·ethanolamine (**8**).

Scheme 3

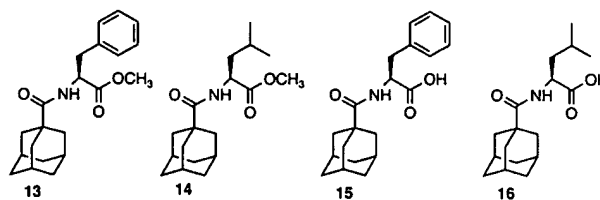


(C-sp³)–B bonds of trialkylboranes including trimethylborane are stable in water to 100 °C.¹¹ The stability of complex **4** in refluxing ethanol during recrystallization attests to the stability of these complexes in polar, protic solvents.

Several diadamantyl compounds structurally similar to complex **4** were prepared. 1-Adamantylcarbonyl chloride was prepared from the reaction of 1-adamantylcarboxylic acid with thionyl chloride, and the acid chloride was subsequently reacted with 1-aminoadamantane to give 1,1-diadamantylamine (**9**) in 19% yield.¹² Additionally, di-1-adamantylamine (**11**) and its hydrochloride salt (**12**) were prepared from 1-bromo-1-adamantane (**10**) according to the procedure of Dervan and McIntyre¹³ (Scheme 3). The reaction of 1-bromo-1-adamantane with 1-aminoadamantane in a thick-walled sealed glass tube (220 °C, 40 h) furnishes **11** after workup with base in a simple, direct procedure. Di-1-adamantylamine (**11**) can be protonated with HCl to furnish the hydrochloride salt **12**.

Several compounds structurally similar to complexes **5** and **7** were also prepared. 1-Adamantylcarbonyl chloride was reacted with L-phenylalanine methyl ester hydrochloride in methylene chloride and triethylamine

(2 equiv) to give amide **13** in 96% yield. Amide **14** was



similarly prepared in 68% yield. Acids **15** and **16** were prepared from the corresponding amides by saponification with lithium hydroxide in 63% and 87% yield, respectively.

Biological Assay Rationale

CD81 is a tetraspanin membrane protein that signals for antiproliferation when bound by antibodies. CD81 has been extensively studied in the immune system¹⁴ where it is implicated in cellular activation,¹⁵ adhesion,¹⁶ proliferation,¹⁷ and differentiation.¹⁸ Tetraspanin–pathogen interactions have been previously reported;¹⁹ however, the significance of these interactions is unknown. Until recently, no ligands that bind CD81

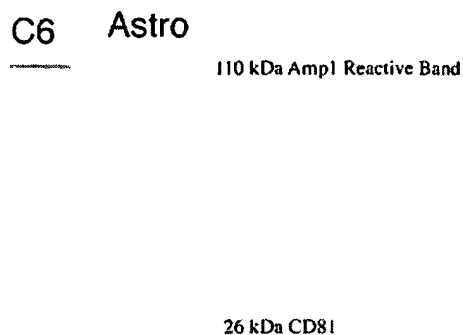


Figure 2. Anti-CD81 Western blot analysis of astrocyte and C6 glioma cell lines.

have been described except for antibodies, many of them antiproliferative, and the HCV-E2 glycoprotein. The importance of the binding of the HCV-E2 glycoprotein to CD81-LEL is not completely understood; however, it is believed to be involved in mediating viral processes of cell entry²⁰ and immune evasion.²¹

The choice of cells for this assay for CD81 binding and activation was based on evidence that rat pup astrocytes up-regulate the expression of CD81 during postnatal days 4–8.¹² This is a contact inhibition mechanism that signals the end of the growth phase in rat glial development. Primary cultures of astrocytes prepared prior to the contact inhibition *in vivo* provide cell culture. The CD81-deficient cell line, C6 glioma, is a commercially available transformed cell line that expresses CD81 at levels that are undetectable by Western blot analysis.²² C6 glioma cells are a transformed cell line derived from glia that has retained many of the properties of astrocytes.²³ The rat glial origin of both these cell lines suggests that the cellular receptors present should be very similar with the notable exception of CD81 (Figure 2). Thus, astrocyte-selective antiproliferative activity provides compelling experimental evidence for a CD81 binding mediated mechanism.

Immunoblots of protein samples were taken from rat astrocytes and rat C6 glioma. The total load of proteins from both samples was balanced. The 110 kDa antigen is recognized in both samples immunoblotted with AMP1 (anti-CD81 antibody). The 26 kDa CD81 band is stained only in the astrocyte band. This indicates CD81 is expressed at significantly lower levels in the C6 cells relative to astrocytes.

An observed reduction in the percent survival of astrocyte versus C6 glioma cells in cell lines treated with a given compound at concentrations varying from 0.1 to 10 μ M constitutes an efficient assay to identify small molecules with strong potential for CD81-mediated antiproliferation. Those compounds showing the most promise in this assay serve as lead compounds in the design of structurally similar analogues for testing. An observed dose-dependence on the percent survival from such a test provides further evidence for the CD81-

mediated antiproliferation model. However, subsequent experiments to provide direct evidence of CD81 binding are necessary for the promising candidates identified in this assay.

Data Analysis

The screening data were collected in three separate experiments of four wells per concentration. Also, the average growth of eight wells containing no test compound was used as a negative control for each microtiter plate. The antiproliferative character of each compound was reported as the percent survival (% survival), calculated as the average A_{560} ratio for treated cells divided by negative control cells, expressed as a percentage. Values less than 100% indicate some degree of antiproliferation. Promising compounds in this assay will display selective antiproliferation in a CD81-enriched cell line (primary astrocyte culture), while the proliferation of CD81-deficient cells (C6 glioma) will not be affected in the presence of an equal concentration of a given compound. The results for compounds **5**, **7**, **8**, **13**, **15**, and **16**, in addition to the reference anti-HCV drug, amantidine, are summarized in Figure 3.

Discussion

In the process of screening over 500 compounds on proliferation of astrocyte cells over C6 glioma cells, the 1-boraadamantaneamine complexes **5**, **7**, and **8** and amantidine analogues **13**, **15**, and **16** proved to be the only compounds that demonstrate astrocyte selective activity. The activity of these compounds was observed in a limited concentration range with nonspecific cytotoxicity at higher concentrations. Among them, compound **15** shows the least nonspecific cytotoxicity and the strongest astrocyte selective activity.

In contrast, many compounds from several structural classes have been observed to be C6 selective.²⁴ This suggests that C6 glioma antiproliferation is easier to achieve because of its clonal, highly mitotic character.²⁵ Amantidine analogues **4**, **6**, **9**, **11**, and **14** show a non-astrocyte selective antiproliferative effect in the 0.1–10 μ M concentration range (Supporting Information).

The neonatal astrocytes by comparison are a heterogeneous collection of glial cell types whose common denominator is the overexpression of CD81 to detect cell–cell contact and signal a developmentally programmed contact inhibition. In accordance with the experimental evidence of the present study, this body of research strongly supports the model that the 1-boraadamantaneamine complexes and amantidine analogues are antiproliferative because of CD81 activation. The identification of these astrocyte-selective antiproliferative compounds provides the stimulus to search for even more potent compounds. At present, analogues **5**, **7**, **8**, **13**, **15**, and **16** are the only known compounds that selectively inhibit astrocyte proliferation without affecting C6 glioma proliferation.

According to the assay, compound **15** displayed the greatest astrocyte-selective antiproliferative character (82% \pm 2% cell survival at 5 μ M). In contrast, the reference anti-HCV drug, amantidine, displayed antiproliferative character at higher concentrations than other analogues of this group. Unfortunately, analogues **5**, **7**, **8**, **13**, **15**, and **16** display nonspecific cytotoxicity

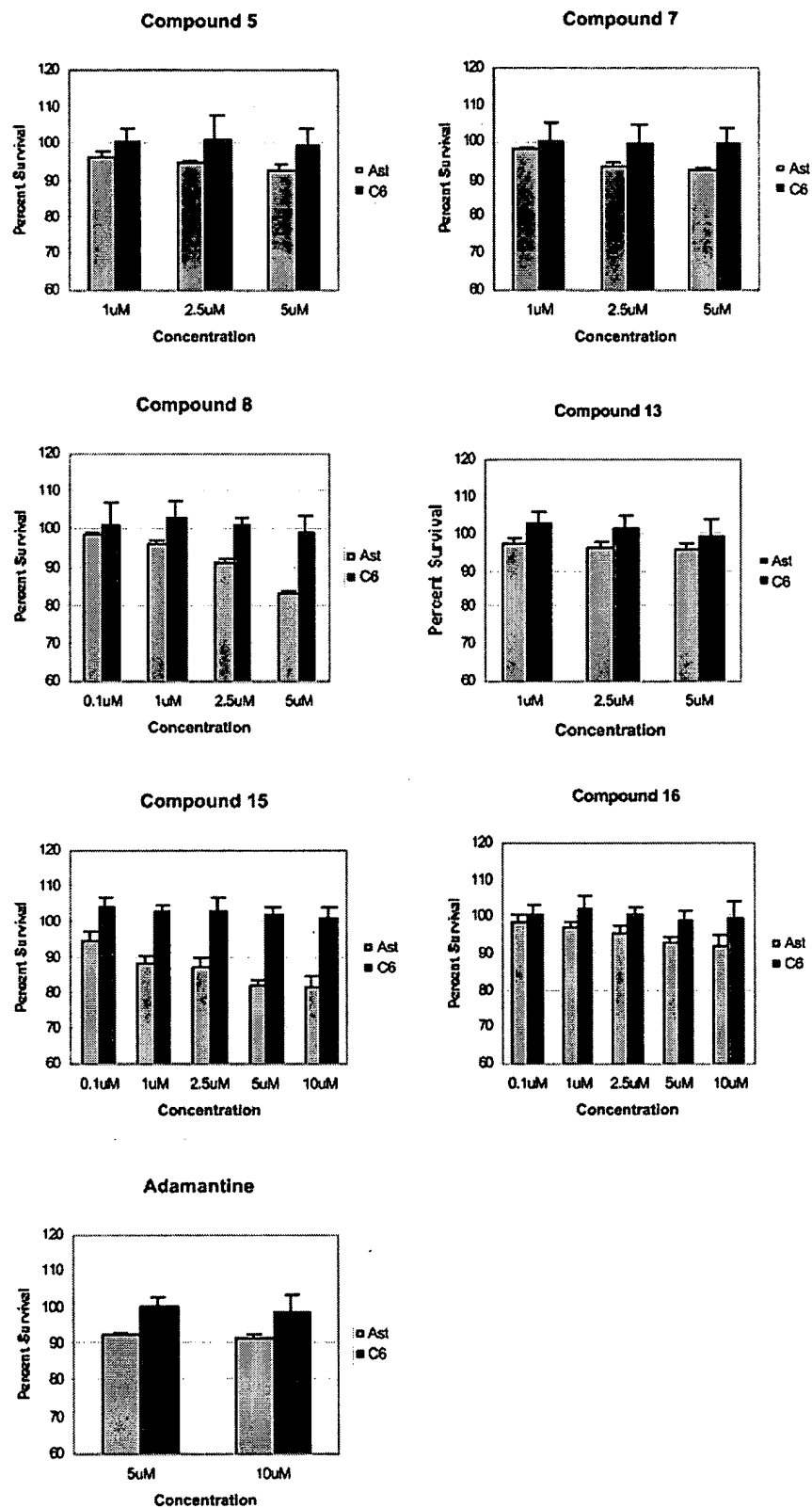


Figure 3. Astrocyte-selective antiproliferative activity of amantidine analogues **5**, **7**, **8**, **13**, **15**, and **16** in addition to amantidine. Three separate dose-response experiments were performed at each concentration. Each experiment consisted of four wells per concentration. The percent survival plotted is the average of all 12 wells of the three separate assays. Error bars represent standard deviation. The graphs were generated using Microsoft Excel.



Figure 4. Three perspectives of the minimized structure showing the CD81-LEL protein binding complex 5. The Thr163 and Phe186 residues of the CD81-LEL are shown in purple, and the 1-boraadamantane-L-phenylalanine (5) is yellow for reference.

at high concentrations ($>10 \mu\text{M}$). We are currently investigating the design of derivatives that lack the nonspecific cytotoxicity based on the lead compounds identified in this paper. The experiments demonstrate the dose-dependent nature of the astrocyte-selective activity of **15** compared to amantidine. This dose-dependent, astrocyte-selective antiproliferative activity is consistent with binding and activation of CD81. By comparison, the standard anti-HCV drug amantidine displayed only a slight reduction in astrocyte proliferation and only at higher concentration. The most promising lead compound, amantidine analogue **15**, is currently being used as the basis for the design of new derivatives in our laboratory for further characterization of this astrocyte-selective antiproliferation.

Modeling

Because of the promising results indicating that complexes **5** and **15** promote a CD81-dependent antiproliferation of astrocyte cell lines, a modeling study was undertaken in an attempt to elucidate a possible site on the CD81-LEL for the binding of complex **5** or other molecules with a 1-aminoadamantane core motif. The atomic coordinates of the human CD81-LEL are available at the Protein Data Bank (PDB) with the accession code 1g8q. The PDB file for the CD81-LEL comprises 176 amino acids and 194 water molecules, and the two chains of the CD81-LEL connected by two disulfide bridges are numbered Phe113-His202 and Phe213-His302 (the C-terminal His residue in both chains is simply a His-tag).

The Thr163 and Phe186, both reported to participate in HCV-E2 binding,²⁶ are positioned on the C-helix and D-helix, respectively. In fact, there is a cleft between the C-helix and D-helix that appears suitable for a competitive binding association of a small molecule with a 1-aminoadamantane core motif. Thus, by use of the Insight II modeling program, 1-aminoadamantane was positioned in the cleft between the C and D helices of the CD81-LEL. The interactions between the cleft and 1-aminoadamantane were minimized by applying a suitable docking algorithm, and a minimized conformation was quickly obtained in which the adamantyl group was buried inside the cleft and the amine group was positioned next to the Thr163 free hydroxyl group. Since the adamantane core fit into the cleft like a ball into a socket, with marginal dead space between the interior

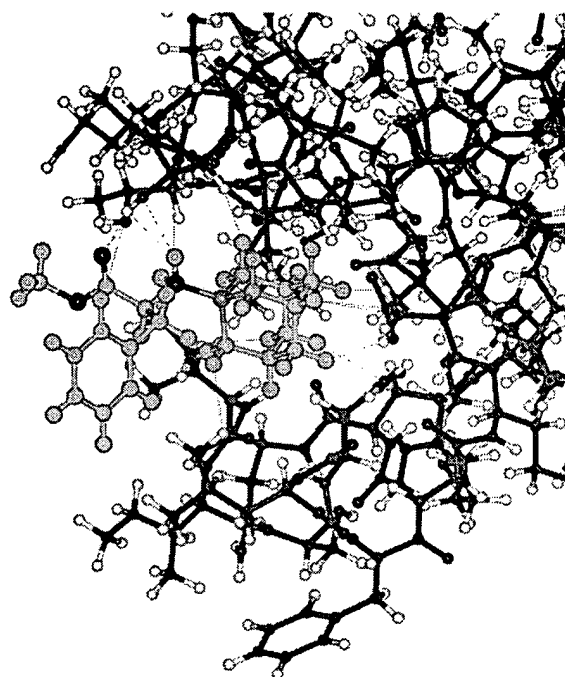


Figure 5. Hydrogen bonding and van der Waals interactions as illustrated by modeling for complex **5** in the active site of CD81.

walls of the cleft and the adamantane framework, we reasoned that other 1-aminoadamantane analogues would bind in a similar fashion. Thus, complex **5** was built in place of the 1-aminoadamantane simply by changing the carbon of the 1-aminoadamantane to boron and building the L-phenylalanine methyl ester off the amine. The docking algorithm was reapplied to minimize interactions between complex **5** and the residues surrounding the cleft. Three perspectives of the resulting minimized structure showing the CD81-LEL protein binding to complex **5** are shown in Figure 4. The Thr163 and Phe186 residues are purple and the 1-boraadamantane-L-phenylalanine (**5**) is yellow for reference.

In the minimized CD81-LEL complex **5** structure, one of the amine hydrogen atoms of complex **5** is almost within hydrogen-bonding distance (2.33 Å) of the oxygen of the Thr163 hydroxyl group. Also, the carbonyl oxygen of the L-phenylalanine methyl ester of **5** is within hydrogen-bonding distance (1.84 Å) of the hydroxyl

proton of the Thr163 residue. Together with these hydrogen-bonding interactions and the van der Waals interactions of the 1-boraadamantyl core in the cleft formed by the C and D helices, we suggest that this is the most probable mode of binding for the 1-boraadamantaneamine complexes to CD81-LEL (Figure 5).

Conclusion

A variety of 1-boraadamantaneamine complexes were synthesized by stoichiometric addition of a primary amine to 1-boraadamantane·THF. These complexes were subsequently evaluated for an antiproliferative effect on CD81-enriched cell lines to provide evidence for binding and activation of the CD81 receptor. Additional amantidine analogues structurally similar to the 1-boraadamantaneamine complexes were also synthesized. Compounds **5**, **7**, **8**, **13**, **15**, and **16** display an antiproliferative effect on the CD81-enriched astrocytes relative to the CD81-deficient cell lines, suggesting that the compounds bind and activate the CD81 receptor over 0.1–10 μ M. However, analogue **15** displayed the greatest astrocyte-selective antiproliferative character in this range of concentration.

These studies demonstrate that a reproducible difference between the percent survival of astrocyte and that of C6 glioma cell lines for a small molecule in micromolar concentrations is consistent with CD81-mediated antiproliferation. The astrocyte-selective antiproliferation, albeit in a limited concentration range of 0.1–10 μ M of analogues **5**, **7**, **8**, **13**, **15**, and **16**, suggests CD81-dependent antiproliferation and establishes the potential of 1-boraadamantaneamine derivatives and analogues derived therefrom as anti-HCV agents. If this finding proves to be true, such 1-boraadamantaneamine derivatives and structurally similar analogues will have the potential to prevent or treat HCV infections by blocking viral attachment, virus-cell fusion, or virus entry into host cells or by countering potential mechanisms of HCV immune evasion. While much remains to be learned about HCV viral attachment and entry, this step in the viral life cycle has proven to be an effective point of attack for combating other viruses including HIV²⁷ and rhinoviruses.²⁸ Compounds **5**, **7**, **8**, **13**, **15**, and **16** are currently being evaluated in a cell-based HCV viral assay to determine the extent to which they inhibit HCV RNA synthesis.

Finally, molecular modeling provides an illustration for how the adamantyl framework of 1-boraadamantaneamine compounds might bind to CD81. Indeed, the ease with which amines can be appended to the 1-boraadamantane framework makes it an ideal substrate for the quick and convenient synthesis of 1-aminoadamantane analogues for evaluation of potential binding to CD81.

Experimental Section

Instrumentation. All ¹H NMR spectra were acquired at 400 or 500 MHz on Bruker spectrometers. Chemical shifts (δ) are listed in ppm against deuterated solvent peaks as an internal reference. Coupling constants (*J*) are reported in hertz, and the abbreviations for splitting include the following: s, single; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broad. All ¹³C NMR spectra were acquired on Bruker instruments at 125.8 MHz. Chemical shifts (δ) are listed in ppm against solvent carbon peaks as an internal

reference. All ¹¹B NMR spectra were acquired at 237 MHz on a Bruker spectrometer. All chemical shifts (δ) are listed in ppm against boron trifluoride etherate as an external reference. Infrared spectra (IR) were assayed on a Perkin-Elmer 1600 series FTIR spectrometer. High-resolution mass spectra (EI, hexanes) were recorded using either a VG 7070e high-resolution mass spectrometer or a Fisons Autospec mass spectrometer. Melting points were assayed on a Thomas-Hoover capillary melting point apparatus.

General Procedures. Tetrahydrofuran, methylene chloride, diethyl ether, and benzene were dried by filtration through alumina according to the procedure described by Grubbs.²⁹ All other solvents were distilled from CaH₂ prior to use. Boron trifluoride etherate (BF₃·Et₂O, Aldrich) was distilled over CaH₂ under reduced pressure (20 mmHg, 65 °C). Allyl bromide (Aldrich) was distilled over CaH₂. All reactions were run in flame-dried glassware under a positive N₂ or argon atmosphere. Removal of volatile solvents transpired under reduced pressure using a Büchi rotary evaporator and is referred to as removing solvents in vacuo. Thin-layer chromatography was conducted on precoated (0.25 mm thickness) silica gel plates with 60F-254 indicator (Merck). Column chromatography was conducted using 230–400 mesh silica gel (E. Merck reagent silica gel 60).

Triallylborane (1). The method of Zakharkin and co-workers was followed to make **1**.¹⁰ To a flame-dried 250 mL round-bottom flask sealed with a rubber septum and equipped with a magnetic stir bar, reflux condenser, and thermometer was transferred solid aluminum granules (12.03 g, 446 mmol) and HgCl_{2(s)} (88 mg, 0.32 mmol). Dry, N₂-purged diethyl ether (60 mL) was added, and the solution was stirred and heated to 35 °C. Allyl bromide (52.1 mL, 602 mmol) was added dropwise under a nitrogen atmosphere. Care must be taken to add the allyl bromide slowly, since the reaction with aluminum is exothermic. The reaction mixture was stirred at reflux in an oil bath set at 67 °C for 3 h. The reaction mixture was then transferred via syringe into a septum-sealed flame-dried 250 mL round-bottom flask containing BF₃·Et₂O (20.0 mL, 142 mmol) under nitrogen. This solution was refluxed at 70 °C for 3 h. The ether was removed by short-path distillation under nitrogen. The residue was then distilled under vacuum (20 mmHg) with a short-path distillation head at a head temperature of 65 °C to give crude triallylborane as a clear, colorless liquid (17.00 g, 78%). ¹¹B NMR (237 MHz, CDCl₃) of this distillate showed a major peak, δ 80.7 (br s), 83%, and minor peaks at δ 50.6 (br s), 11%, and δ 32.0 (br s), 6%. The peak at δ 80.7 (br s) has been assigned to triallylborane. On the basis of chemical shift, the peak at δ 50.6 has been tentatively assigned to a borinic ester impurity, most likely the product of triallylborane's reaction with oxygen. A second vacuum distillation (20 mmHg) yielded pure triallylborane as a clear, colorless liquid (13.07 g, 60%): ¹H NMR (500 MHz, CDCl₃) δ 2.2 (br s, 6H), 4.9 (br s, 6H), 5.89–5.98 (p, *J* = 10.6, 3H); ¹³C NMR (125.8 MHz, CDCl₃) δ 134.8, 114.7 (br s), 34.4 (br s); ¹¹B NMR (237 MHz, CDCl₃) δ 80.6 (br s); IR (neat) 3076, 2999, 2973, 2914, 1807, 1635, 1420, 1370, 1270, 1170, 993, 900 cm⁻¹.

3-Methoxy-7-(methoxymethyl)-3-borabicyclo[3.3.1]non-6-ene (2). The basic procedure of Mikhailov and co-workers was followed to synthesize **2**.^{9b} To a flame-dried, two-neck 50 mL flask with stir bar and condenser was added distilled triallylborane (8.5 g, 63 mmol) under a nitrogen atmosphere. The flask was heated with stirring under nitrogen at 130 °C in a preheated oil bath (10 min.). Methyl propargyl ether (4.5 mL, 64 mmol) was added via syringe over the course of 10 min to the stirring triallylborane. The reaction mixture was heated at 130 °C for 1 h. The reaction solution was cooled to room temperature, and methanol (2.9 mL, 72 mmol) was added dropwise under nitrogen with stirring. The reaction was exothermic and accompanied by the evolution of propylene gas. After an additional 5 min of stirring, the reaction mixture was distilled under vacuum (0.10 mmHg) with a short-path distillation head at a head temperature of 79 °C to give NMR-pure 3-methoxy-7-(methoxymethyl)-3-borabicyclo[3.3.1]non-6-ene **6**

(10.75 g, 87%) as a clear, viscous liquid: ^1H NMR (500 MHz, CDCl_3) δ 5.66 (br s, 1H), 3.74 (d, $J = 11.7$, 1H), 3.64 (d, 11.8), 3.58 (s, 3H), 3.19 (s, 3H), 2.49 (br s, 1H), 2.43 (br s, 1H), 2.25 (d, $J = 17.9$, 1H), 1.74 (d, $J = 12.7$, 1H), 1.70 (d, $J = 17.8$, 1H), 1.60 (d, $J = 12.7$, 1H), 1.04 (d, $J = 17.4$, 1H), 0.96 (dd, $J = 17$, 7.5, 1H), 0.86 (d, $J = 17.4$, 1H), 0.83 (dd, $J = 17$, 5.3, 1H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 131.7, 130.8, 77.0, 56.9, 53.0, 34.9, 32.5, 29.0, 26.9, 25.4 (br s), 24.1 (br s); ^{11}B NMR (237 MHz, CDCl_3) δ 55.4 (br s); IR (thin film) 3397, 2909, 1465, 1431, 1371, 1298, 1167, 1101, 1038, 1019, 996, 955, 914, 880, 847, 831, 678 cm^{-1} ; HRMS (EI/hexanes) m/z calcd for $\text{C}_{11}\text{H}_{19}\text{BO}_2$ (M^+) 194.1478, found 194.1481.

1-Boraadamantane-THF (3). The protocol of Mikhailov and co-workers was followed to synthesize **3**.^{9a} To a flame-dried, two-neck 50 mL flask with a stir bar and condenser was added 3-methoxy-7-(methoxymethyl)-3-borabicyclo[3.3.1]non-6-ene (2.8698 g, 14.8 mmol). A solution of borane THF (14.80 mL, 1 M, 14.8 mmol) was slowly added with stirring. The reaction was exothermic, and the mixture came to reflux during addition of the borane-THF. The reaction solution was then lowered into an oil bath preheated to 80 °C and refluxed for 1 h. The THF was removed by distillation at atmospheric pressure under nitrogen, followed by vacuum distillation (20 mmHg). The white residue was then sublimed under vacuum (0.100 mmHg, 70 °C) onto an ice/water chilled coldfinger to yield 1-boraadamantane-THF **3** (1.6140 g, 53%) as a solid, white, crystalline compound: mp 89–91 °C; ^1H NMR (500 MHz, C_6D_6) δ 3.34 (t, $J = 6.8$, 4H), 2.74 (br s, 3H), 2.00 (m, 6H), 1.01 (m, 10H); ^{13}C NMR (125.8 MHz, C_6D_6) δ 68.4, 40.7, 34.6, 29.7 (br s), 24.3; ^{11}B NMR (237 MHz, CDCl_3) δ 5.10 (br s); IR (KBr) 2868, 1438, 1350, 1299, 1252, 1226, 1192, 1119, 1078, 1025, 982, 930, 918, 855, 728, 614 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{13}\text{H}_{24}\text{BO}$ ($M + \text{H}^+$) 207.1923, found 207.1929.

1-Boraadamantane-1-aminoadamantane (4). The procedure of Mikhailov and co-workers was followed to make complex **4**.^{9a} In a 100 mL flask, 1-aminoadamantane (1.05 g, 6.9 mmol) was dissolved in methylene chloride (15.0 mL). In a 25 mL flask, sublimed 1-boraadamantane THF (1.43 g, 6.9 mmol) was dissolved in methylene chloride (10.0 mL), and this solution was added to the 1-aminoadamantane solution with stirring. The solvent was removed in vacuo to yield crude 1-boraadamantane-1-aminoadamantane **4** (1.78 g, 90%) as a fine off-white powder. The 1-boraadamantane-1-aminoadamantane was recrystallized in a minimum amount of ethanol to yield **4** (0.76 g, 38%) as clear, white crystals: mp 190–194 °C; ^1H NMR (500 MHz, CDCl_3) δ 2.63 (br s, 1H), 2.11 (br s, 6H), 1.98 (br s, 1H), 1.83 (br s, 6H), 1.63 (m, 10H), 1.52 (m, 3H), 0.57 (m, 6H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 54.9, 43.4, 40.2, 35.8, 33.4 (br), 33.0, 29.4; IR (KBr) 3275, 3228, 2905, 1567, 1455, 1303, 1231, 1072, 1013, 989, 938, 783, 763 cm^{-1} ; HRMS (FAB) m/z calcd for $\text{C}_{19}\text{H}_{32}\text{BN}$ (M^+) 285.2628, found 285.2609. Anal. Calcd for $\text{C}_{19}\text{H}_{32}\text{BN}$: C, 79.99; H, 11.31; N, 4.91. Found: C, 79.84; H, 11.38; N, 4.91.

1-Boraadamantane-L-Phenylalanine Methyl Ester (5). L-Phenylalanine methyl ester hydrochloride (1.80 g, 8.35 mmol) was dissolved in methylene chloride (25 mL), and this solution was extracted twice with an equal volume of saturated aqueous sodium bicarbonate. The organic layer was dried over sodium sulfate, the solution was filtered, and solvent was removed in vacuo to give L-phenylalanine methyl ester (1.00 g, 67%). L-Phenylalanine methyl ester (0.9079 g, 5.1 mmol) was dissolved in methylene chloride (10.0 mL) in a 100 mL flask. In a 25 mL flask, sublimed 1-boraadamantane-THF (1.0442 g, 5.1 mmol) was dissolved in methylene chloride (10 mL), and this solution was added with stirring to the L-phenylalanine solution. The solvents were removed in vacuo to give 1-boraadamantane-L-phenylalanine methyl ester **5** (1.6 g, 5.1 mmol) as a white crystalline solid: mp 53–59 °C; $[\alpha]_D^{25} +5.7$ (c 0.92, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.38 (m, 3H), 7.13 (d, $J = 6.9$, 2H), 3.99 (m, 1H), 3.89 (m, 1H), 3.85 (s, 3H), 3.17 (dd, $J = 14.3$, 5, 1H), 2.97 (dd, $J = 14.3$, 7.95, 1H), 2.91 (m, 1H), 2.09 (br s, 3H), 1.57 (d, $J = 12.0$, 3H), 1.44 (d, $J = 11.1$, 3H), 0.35 (m, 3H), 0.27 (m, 3H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 172.1, 133.6, 129.5, 129.3, 128.2, 53.2, 53.0, 40.0, 38.3,

32.5, 25.6 (br s); ^{11}B NMR (237 MHz, CDCl_3) δ -7.0; IR (neat) 3282, 3220, 2850, 1738, 1563, 1497, 1445, 1405, 1254, 1078, 989, 941, 855, 838, 779, 747, 701 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{19}\text{H}_{29}\text{BO}_2\text{N}$ ($M + \text{H}^+$) 314.2291, found 314.2266. Anal. Calcd for $\text{C}_{19}\text{H}_{29}\text{BO}_2\text{N}$: C, 72.85; H, 9.01; N, 4.47. Found: C, 72.62; H, 8.99; N, 4.48.

1-Boraadamantane-L-Cysteine Methyl Ester (6). L-Cysteine methyl ester hydrochloride (5.62 g, 32.6 mmol) was dissolved in deionized water (43.0 mL) containing sodium bicarbonate (4.86 g, 57.7 mmol), and the solution was saturated in sodium chloride. The aqueous solution was extracted with methylene chloride, and the organic layer was washed with saturated sodium chloride and dried over sodium sulfate. The methylene chloride was removed in vacuo to afford L-cysteine methyl ester (4.04 g, 29.9 mmol) as a clear, colorless liquid in 91% yield. To a solution of L-cysteine methyl ester (1.0 g, 7.4 mmol) in methylene chloride (10.0 mL) was added a solution of 1-boraadamantane-THF **3** (1.4694 g, 7.1 mmol) in methylene chloride (10.0 mL) with stirring. The methylene chloride was removed in vacuo to afford 1-boraadamantane-L-cysteine methyl ester **6** (1.9 g, 7.1 mmol) as a colorless crystalline solid: mp 109–111 °C; $[\alpha]_D^{25} -11.2$ (c 0.79, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 3.99 (m, 1H), 3.88 (m, 1H), 3.84 (s, 3H), 3.76 (br s, 1H), 3.04 (dd, $J = 9.1$, 4.6, 2H), 2.13 (br s, 3H), 1.59 (m, 3H), 1.48 (m, 3H), 1.29 (t, $J = 9.1$, 1H), 0.44 (br s, 6H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 53.7, 40.1, 32.7, 30.4, 26.7; ^{11}B NMR (237 MHz, CDCl_3) δ -8.4 (br s); IR (KBr) 3422, 3280, 3235, 2856, 1734, 1552, 1439, 1388, 1281, 1232, 1205, 1177, 1076, 988 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{13}\text{H}_{25}\text{BO}_2\text{NS}$ ($M + \text{H}^+$) 270.1702, found 270.1692. Anal. Calcd for $\text{C}_{13}\text{H}_{25}\text{BO}_2\text{NS}$: C, 58.00; H, 8.99; N, 5.20. Found: C, 58.21; H, 9.05; N, 5.27.

1-Boraadamantane-L-Leucine Methyl Ester (7). L-Leucine methyl ester hydrochloride (5.95 g, 32.8 mmol) was dissolved in deionized water (43.0 mL) containing sodium bicarbonate (4.86 g, 57.7 mmol), and the solution was saturated in sodium chloride. The aqueous solution was extracted with methylene chloride, and the organic layer was washed with saturated sodium chloride and dried over sodium sulfate. The methylene chloride was removed in vacuo to afford L-leucine methyl ester (4.26 g, 29.3 mmol) as a clear, colorless liquid in 90% yield. To a solution of L-leucine methyl ester (1.06 g, 7.36 mmol) in methylene chloride (10.0 mL) was added a solution of 1-boraadamantane-THF **3** (1.50 g, 7.29 mmol) in methylene chloride (10 mL) with stirring. The methylene chloride was removed in vacuo to afford 1-boraadamantane-L-leucine methyl ester **7** (1.96 g, 7.02 mmol) as a white crystalline solid in 96% yield: mp 91–93 °C; $[\alpha]_D^{25} -2.0$ (c 0.88, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 4.14 (m, 1H), 3.80 (s, 3H), 3.74 (dd, $J = 8.5$, 11.7, 1H), 2.83 (d, $J = 11.7$, 1H), 2.11 (br s, 3H), 1.62 (m, 2H), 1.58 (d, $J = 12.3$, 3H), 1.52 (d, $J = 8.5$, 1H), 1.48 (d, $J = 11.2$, 3H), 0.98 (t, $J = 5.7$, 6H), 0.42 (d, $J = 10.5$, 3H), 0.35 (d, $J = 10.5$, 3H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 53.2, 51.1, 42.8, 40.2, 32.7, 30.1, 25.1, 22.8, 22.0; ^{11}B NMR (237 MHz, CDCl_3) δ -4.7; IR (KBr) 3308, 3242, 2865, 1733, 1560, 1438, 1388, 1288, 1252, 1219, 1148, 1071, 989, 942, 789 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{16}\text{H}_{31}\text{BO}_2\text{N}$ ($M + \text{H}^+$) 280.2451, found 280.2450. Anal. Calcd for $\text{C}_{16}\text{H}_{31}\text{BO}_2\text{N}$: C, 68.82; H, 10.83; N, 5.02. Found: C, 68.99; H, 11.2; N, 5.06.

1-Boraadamantane-Ethanolamine (8). To a solution of ethanolamine (0.356 g, 5.8 mmol) in methylene chloride (10.0 mL) was added a solution of 1-boraadamantane-THF **3** (1.20 g, 5.8 mmol) in methylene chloride (10.0 mL) with stirring. The methylene chloride was removed in vacuo to afford 1-boraadamantane-ethanolamine **8** (1.14 g, 5.8 mmol) as a white, crystalline solid in quantitative yield: mp 131–133 °C; ^1H NMR (500 MHz, CDCl_3) δ 3.78 (t, $J = 4.9$, 2H), 3.15 (br s, 2H), 2.87 (m, 2H), 2.14 (br s, 3H), 1.62 (d, $J = 11.6$, 3H), 1.51 (d, $J = 11.9$, 3H), 0.44 (br s, 6H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 60.8, 41.9, 40.8, 32.8, 30.5 (br s); ^{11}B NMR (237 MHz, CDCl_3) δ -7.0; IR (KBr) 3610, 3425, 3289, 3247, 2861, 1587, 1231, 1068 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{11}\text{H}_{23}\text{BNO}$ ($M + \text{H}^+$) 195.1797, found 195.1791. Anal. Calcd for $\text{C}_{11}\text{H}_{23}\text{BNO}$: C, 67.72; H, 11.37; N, 7.18. Found: C, 67.47; H, 11.43; N, 7.32.

Di-1-adamantylamide (9). To a solution of 1-adamantyl-carbonyl chloride (3.31 g, 16.6 mmol) in CH_2Cl_2 (16.0 mL) at 0 °C were added 1-aminoadamantane (2.52 g, 16.7 mmol) and pyridine (4.0 mL, 49.5 mmol). The reaction mixture was warmed to room temperature and stirred (3 h). The reaction was quenched by washing with 10% HCl (25.0 mL) and saturated NaCl (25.0 mL). The organic layer was separated, dried over sodium sulfate, and filtered, and excess CH_2Cl_2 was removed in vacuo. The crude product was chromatographed (SiO_2 , 2:1 hexane/EtOAc) to give **9** as a white solid (1.0 g, 19%): mp 314–315 °C; ^1H NMR (500 MHz, CDCl_3) δ 5.22 (br s, 1H), 2.06 (m, 6H), 1.98 (d, J = 3.0, 6H), 1.80 (d, J = 2.6, 6H), 1.68 (m, 12H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 177.2, 51.2, 41.7, 41.6, 40.9, 39.4, 36.6, 36.4, 29.5, 28.2; IR (KBr) 3328, 2902, 1637, 1540, 1449 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{21}\text{H}_{32}\text{NO}$ (M)⁺ 313.2405, found 313.2406. Anal. Calcd for $\text{C}_{21}\text{H}_{31}\text{NO}$: C, 80.46; H, 9.97; N, 4.47. Found: C, 80.59; H, 9.97; N, 4.49.

Di-1-adamantylamine (11). Di-1-adamantylamine was synthesized according to the protocol of Dervan and McIntyre. A thick-walled glass tube, closed at one end and narrowed at the other, was filled with 1-adamantylamine (7.4 g, 49 mmol) and 1-adamantyl bromide (5.52 g, 26 mmol). After the narrow neck was sealed under vacuum, the tube was placed in a ceramic heating oven and heated to 220 °C for 40 h. The solid melted and then solidified after 1 day. Upon cooling to room temperature, the crude product was crushed with a mortar and pestle and extracted with diethyl ether (250 mL) and 25% NaOH (250 mL) by shaking vigorously in a separatory funnel. The ether layer was separated and shaken with 2.4 N HCl (250 mL) to precipitate di-1-adamantylamine as the hydrochloride salt. The precipitate was filtered and washed with water (50 mL). The resulting white solid was shaken with a two-phase layer of ether (200 mL) and 25% NaOH (250 mL) to regenerate di-1-adamantylamine in ether. The ether layer was separated and dried over anhydrous K_2CO_3 . After filtration and removal of the solvent in vacuo, 1.87 g (26%) of di-1-adamantylamine (**11**) was obtained as a fluffy white solid: mp 194–195 °C (lit. 196–197 °C); ^1H NMR (500 MHz, CDCl_3) δ 2.01 (br s, 6H), 1.76 (br d, 12H), 1.61 (br s, 12H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 52.6, 46.5, 36.6, 30.0; IR (KBr) 2898, 1448, 1342, 1304, 1160 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{20}\text{H}_{32}\text{N}$ (M)⁺ 285.2456, found 285.2453. Anal. Calcd for $\text{C}_{20}\text{H}_{31}\text{N}$: C, 84.15; H, 10.95; N, 4.91. Found: C, 83.92; H, 10.98; N, 4.95.

Hydrochloride (12). Anal. Calcd for $\text{C}_{20}\text{H}_{32}\text{NCl}$: C, 74.62; H, 10.02; N, 4.35; Cl, 11.01. Found: C, 74.55; H, 10.10; N, 4.36; Cl, 11.06.

(S)-2-[(Adamantane-1-carbonyl)amino]-3-phenylpropionic Acid Methyl Ester (13). 1-Adamantanecarboxylic acid (1.09 g, 6.0 mmol) was dissolved in thionyl chloride (15.0 mL, 206 mmol), and the mixture was heated to reflux (1 h) under nitrogen. Excess thionyl chloride was removed in vacuo, and the crude 1-adamantanecarbonyl chloride was dissolved in benzene (10 mL). The solution of 1-adamantanecarbonyl chloride was added to a solution of L-phenylalanine methyl ester hydrochloride (1.30 g, 6.0 mmol) and triethylamine (1.68 mL, 12.0 mmol) in benzene (40 mL) at 0 °C with stirring. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was filtered to remove triethylamine hydrochloride, and excess solvents were removed in vacuo. The crude product was chromatographed (SiO_2 , 2:1 hexane/EtOAc) to give **13** (1.99 g, 96%) as a white solid: mp 98–98.5 °C; $[\alpha]_D^{25}$ +69.9 (c 0.74, CHCl_3), lit. $[\alpha]_D^{25}$ +63.7;³⁰ ^1H NMR (500 MHz, CDCl_3) δ 7.25 (m, 3H), 7.08 (d, J = 6.9, 2H), 6.02 (br d, J = 7.2, 1H), 4.87 (q, J = 5.7, 1H), 3.73 (s, 3H), 3.12 (dq, J = 13.8, 5.7, 2H), 2.02 (br s, 3H), 1.79 (m, 6H), 1.72 (m, 6H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 177.3, 172.3, 135.9, 129.3, 128.5, 127.1, 52.7, 52.3, 40.6, 39.0, 37.8, 36.4, 28.0; IR (KBr) 3318, 2901, 1751, 1639, 1534 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{21}\text{H}_{27}\text{NO}_3$ (M)⁺ 341.1991, found 341.1993. Anal. Calcd for $\text{C}_{21}\text{H}_{27}\text{NO}_3$: C, 73.87; H, 7.97; N, 4.10. Found: C, 73.57; H, 7.94; N, 4.24.

(S)-2-[(Adamantane-1-carbonyl)amino]-4-methylpentanoic Acid Methyl Ester (14). 1-Adamantanecarboxylic

acid (1.09 g, 6.0 mmol) was dissolved in thionyl chloride (15.0 mL, 206 mmol) and heated to reflux (1 h) under nitrogen. Excess thionyl chloride was removed in vacuo, and the crude 1-adamantanecarbonyl chloride was dissolved in benzene (10 mL). The solution of 1-adamantanecarbonyl chloride was added to a solution of L-leucine methyl ester hydrochloride (1.09 g, 6.0 mmol) and triethylamine (1.68 mL, 12.0 mmol) in benzene (40 mL) at 0 °C with stirring. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was filtered to remove triethylamine hydrochloride, and excess solvents were removed in vacuo. The crude product was chromatographed (SiO_2 , 2:1 hexane/EtOAc) to give **14** (1.26 g, 68%) as a white solid: mp 137–138 °C; $[\alpha]_D^{25}$ –2.2 (c 0.38, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 5.96 (m, 1H), 4.63 (m, 1H), 3.72 (s, 3H), 2.05 (s, 3H), 1.87 (m, 6H), 1.61 (m, 6H), 1.54 (m, 3H), 0.94 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 177.7, 173.9, 52.2, 50.4, 41.8, 40.6, 39.1, 36.5, 28.1, 24.9, 22.8, 22.1; IR (KBr) 3342, 2913, 1747, 1635, 1533 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{18}\text{H}_{30}\text{NO}_3$ (M + H)⁺ 308.2226, found 308.2221. Anal. Calcd for $\text{C}_{18}\text{H}_{29}\text{NO}_3$: C, 70.32; H, 9.51; N, 4.56. Found: C, 70.04; H, 9.49; N, 4.69.

(S)-2-[(Adamantane-1-carbonyl)amino]-3-phenylpropionic Acid (15). To a solution of amide **14** (0.43 g, 1.3 mmol) dissolved in THF (1.2 mL) was added a solution of lithium hydroxide (0.076 g, 1.8 mmol) in water (1.2 mL). The reaction mixture was stirred for 1 h and acidified with HCl, and excess solvents were removed in vacuo. The residue was chromatographed (SiO_2 , 95:5 chloroform/MeOH) to give **15** (0.26 g, 63%) as a solid white foam: mp 65–67 °C; $[\alpha]_D^{25}$ +46.8 (c 0.47, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.29 (m, 3H), 7.15 (d, J = 7.1, 2H), 6.06 (d, J = 6.3, 1H), 4.81 (q, J = 6.1, 1H), 3.20 (m, 2H), 2.0 (s, 3H), 1.76 (m, 6H), 1.7 (m, 6H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 178.7, 174.5, 135.7, 129.4, 128.6, 127.2, 53.1, 40.6, 38.9, 37.0, 36.3, 27.9; IR (KBr) 3423, 2906, 1735, 1624, 1522 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{20}\text{H}_{25}\text{NO}_3$ (M)⁺ 327.1834, found 327.1833. Anal. Calcd for $\text{C}_{20}\text{H}_{25}\text{NO}_3$: C, 73.37; H, 7.70; N, 4.28. Found: C, 73.61; H, 7.97; N, 4.15.

(S)-2-[(Adamantane-1-carbonyl)amino]-4-methylpentanoic Acid (16). To a solution of amide **13** (0.24 g, 0.78 mmol) dissolved in THF (1.2 mL) was added a solution of lithium hydroxide (0.052 g, 1.24 mmol) in water (1.2 mL). The reaction mixture was stirred for 1 h and acidified with HCl, and excess solvents were removed in vacuo. The residue was chromatographed (SiO_2 , 95:5 chloroform/MeOH) to give **16** (0.20 g, 87%) as a white powder: mp 173–174 °C; $[\alpha]_D^{25}$ –14.5 (c 0.36, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 9.15 (br s, 1H), 6.06 (br s, 1H), 4.59 (q, J = 5.6, 1H), 2.04 (s, 3H), 1.86 (m, 6H), 1.65 (m, 8H), 1.58 (m, 1H), 0.94 (m, 6H); ^{13}C NMR (125.8 MHz, CDCl_3) δ 178.7, 176.6, 50.8, 41.0, 40.6, 38.9, 36.4, 28.0, 24.9, 22.8, 22.0; IR (KBr) 3402, 3100 (br), 2911, 1735, 1626, 1542 cm^{-1} ; HRMS (CI) m/z calcd for $\text{C}_{17}\text{H}_{27}\text{O}_3\text{N}$ (M)⁺ 293.1991, found 293.1987. Anal. Calcd for $\text{C}_{17}\text{H}_{27}\text{NO}_3$: C, 69.59; H, 9.28; N, 4.77. Found: C, 69.93; H, 9.54; N, 4.52.

Molecular Modeling. General Methodology. All molecular modeling studies were done on a Silicon Graphics computer running the Insight II (version 97)³¹ and Discover (version 2.98) software (Molecular Simulations, Inc., San Diego, CA). Minimization methods were employed and refined to the local minima of the structures as described below. The docking between the adamantane derivatives and the enzyme was carried out manually and then Assembly refined in the Discover III mode employing an all multipole summation method with a dielectric constant of 1.0. The potentials employed were the ESFF (Extensible Systematic Force Field) in the Insight package. Conjugate (Polak–Ribiere) was used in energy minimizations. The maximum derivative was 0.899 with a total potential energy of –4095.7 kcal/mol.

Cell Culture. The CD81-enriched astrocytes as described by Geisert et al. were derived from rat pup glia primary culture collected 4–8 days postpartum.³² The primary cultures were prepared as previously described by McCarthy et al.³³ C6 glioma is a CD81-deficient cell line that was derived from a frozen rat cell culture bought from ATCC. The cells were maintained in 10% fetal calf serum (FCS, Hyclone) in Basal

Medium Eagle (BME, Gibco) in 75 cm² flasks. The cells were grown for several weeks before use in screening assays.

Screening Assays. All samples were dissolved completely to make a 100 μ M stock solution in a 1% DMSO HBSS and diluted to produce a series of concentrations. A 10 μ L aliquot of these initial solutions was added to 190 μ L of 2% FCS-BME to produce the test concentration. The final concentration of DMSO was 0.05%, and it showed no effect on the cell proliferation. The vehicle solution was tested as a control. Dilutions were performed such that cosolvent concentrations did not vary for a particular experiment. The assays were performed with parallel manipulations of astrocyte and C6 glioma cells. The cells were trypsinized and transferred to 96-well microtiter plates at a cell density of 10³ C6 cells per well and 3 \times 10³ astrocytes per well. The optimal cell seeding density on cell growth was determined by doing identical experiments at different cell densities. The range of cell densities used in screening assays did not cause variability in the growth rates of C6 or primary astrocyte cultures. The experimental treatment of the cells was incubation with test compound for 4 days at 37 $^{\circ}$ C, 5% CO₂. The growth kinetics did not show variability for the 4-day culture period for C6 and astrocytes.

After the incubation period, cells were fixed with 4% formaldehyde for 30 min. Fixative was removed by three isotonic saline washes of 5 min duration. Cells were quantitated spectrophotometrically using 0.1% cresylect violet stain prepared in a sodium acetate buffer, pH 3.7, as described previously by Kueng et al.³⁴ Briefly, the cells were dyed with 100 μ L of the dye for 30 min. The excess dye was rinsed from the wells with two 200 μ L portions of normal saline. The dye was released from the cells using a 100 μ L aliquot of 10% glacial acetic acid for 20 min. The absorbance of the resulting supernatant was measured using a Titertech Pro Plus microtiter plate reader using a 560 μ m wavelength filter.

Immunoblot Method. Samples containing equal amounts of protein were dissolved in nonreducing sample buffer (2% SDS, 10% glycerol in 0.05 M Tris-HCl buffer, pH 6.8) and run on 10% SDS-PAGE. After electrophoresis, the gels were transferred to nitrocellulose for immunoblot analysis. Then the blots were blocked in nonfat dry milk, probed with AMP1 (CD81 antibody) as a primary antibody, rinsed in borate buffer, pH 8.5, incubated in HRP-labeled secondary antibody, and reacted with diaminobenzidine and hydrogen peroxide. The level of immunoreaction product was determined by scanning the blots and analyzing these scans with the National Institutes of Health image program.

Acknowledgment. This research was supported by grants from the National Science Foundation (Grant CHE-9617475) and PHS (Grant R01EY12369). C.E.W. thanks Allergan, Inc. for a graduate fellowship.

Supporting Information Available: X-ray data and other data for compounds 4, 6, 9, 11, and 14. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Cohen, J. The Scientific Challenge of Hepatitis C. *Science* **1999**, *285*, 26–30.
- (2) McHutchison, J. G.; Gordon, S. C.; Schiff, E. R.; Shiffman, M. L.; Lee, W. M.; Rustgi, V. K.; Goodman, Z. D.; Ling, M.; Cort, S.; Albrecht, J. K. Interferon Alfa-2b Alone or in Combination with Ribavirin as Initial Treatment for Chronic Hepatitis C. *N. Engl. J. Med.* **1998**, *339*, 1485–1492.
- (3) (a) Chan, J.; O'Riordan, K.; Wiley, T. E. Amantidine's Viral Kinetics in Chronic Hepatitis C Infection. *Dig. Dis. Sci.* **2002**, *47*, 438–442. (b) Tabone, M.; Laudi, C.; Delmastro, B.; Bigliano, A.; Andreoni, M.; Chieppa, F.; Bonardi, R.; Cariti, G.; Cusumano, S.; Brunello, F.; Calleri, G.; Manca, A.; Della Monica, P. D.; Sidoli, L.; Rizzetto, M.; Pera, A. Interferon and Amantidine in Combination as Initial Treatment for Chronic Hepatitis C Patients. *J. Hepatol.* **2001**, *35*, 517–521. (c) Mangia, A.; Minerva, N.; Annese, M.; Leandro, G.; Villani, M. R.; Santoro, R.; Carretta, V.; Bacca, D.; Giangaspero, A.; Bisceglia, M.; Ventrella, F.; Dell'Erba, G.; Andriulli, A. A Randomized Trial of Amantidine and Interferon versus Interferon Alone as Initial Treatment for Chronic Hepatitis C. *Hepatology* **2001**, *33*, 989–993. (d) Brilanti, S.; Levantesi, F.; Masi, L.; Foli, M.; Bolondi, L. Triple Antiviral Therapy as a New Option for Patients with Interferon Nonresponsive Chronic Hepatitis C. *Hepatology* **2000**, *32*, 630–634. (e) Smith, J. P. Treatment of Chronic Hepatitis C with Amantidine. *Dig. Dis. Sci.* **1997**, *42*, 1681–1687.
- (4) Priestley, E. S.; Decicco, C. P. 1-Aminocyclopropaneboronic Acid: Synthesis and Incorporation into an Inhibitor of Hepatitis C Virus NS3 Protease. *Org. Lett.* **2000**, *2*, 3095–3097.
- (5) Pileri, P.; Uematsu, Y.; Campagnoli, S.; Galli, G.; Falugi, F.; Petracca, R.; Weiner, A. J.; Houghton, M.; Rosa, D.; Grandi, G.; Abrignani, S. Binding of Hepatitis C Virus to CD81. *Science* **1998**, *282*, 938–941.
- (6) (a) Kitadokoro, K.; Galli, G.; Petracca, R.; Falugi, F.; Grandi, G.; Bolognesi, M. Crystallization and Preliminary Crystallographic Studies on the Large Extracellular Domain of Human CD81, A Tetraspanin Receptor for Hepatitis C. *Acta Crystallogr., Sect. D* **2001**, *57*, 156–158. (b) Kitadokoro, K.; Galli, G.; Petracca, R.; Falugi, F.; Grandi, G.; Bolognesi, M. CD81 Extracellular Domain 3D Structure: Insight into the Tetraspanin Superfamily Structural Motifs. *EMBO J.* **2001**, *20*, 12–18.
- (7) Petracca, R.; Falugi, F.; Galli, G.; Norais, N.; Rosa, D.; Campagnoli, S.; Burgio, V.; Di Stasio, E.; Giardina, B.; Houghton, M.; Abrignani, S.; Grandi, G. Structure Function Analysis of Hepatitis C Virus Envelope-CD81 Binding. *J. Virol.* **2000**, *74*, 4824–4830.
- (8) (a) Lagutkin, N. A.; Mitin, N. I.; Zubairov, M. M.; Arkhipova, T. N.; Petracheva, T. K.; Mikhailov, B. M.; Smirnov, V. N.; Baryshnikova, T. K.; Govorov, N. N. Synthesis of Nitrogen-Containing Complexes of 1-Boraadamantane and Their Antiviral Activity. *Khim.-Farm. Zh.* **1983**, *17*, 1077–1080. (b) Mikhailov, B. M.; Smirnov, V. N.; Smirnova, O. D.; Kasparov, V. A.; Lagutkin, N. A.; Mitin, N. I.; Zubairov, M. M. Synthesis and Antiviral Activity of 1-Boraadamantane Complexes. *Khim.-Farm. Zh.* **1979**, *13*, 35–39.
- (9) (a) Mikhailov, B. M.; Baryshnikova, T. K.; Kiselev, V. G.; Shashkov, A. S. Synthesis of 1-Boraadamantane and Its Complexes by Use of 3-Methoxy-7-methoxymethyl-3-borabicyclo[3.3.1]non-6-ene. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1979**, *28*, 2544–2551. (b) Mikhailov, B. M.; Baryshnikova, T. K. Reaction of Triallylborane with Propargyl Alkyl Ethers. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1979**, *11*, 2541–2544.
- (10) Zakharkin, L. I.; Stanko, V. I. Simple Synthesis of Triallylborane and Some of Its Conversions. *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk* **1960**, *10*, 1896–1898.
- (11) (a) Ulmschneider, D.; Goubeau, J. Reaction of Trimethylboranes. *Chem. Ber.* **1957**, *90*, 2733–2738. (b) Mikhailov, B. M.; Bubnov, Yu. N. *Organoboron Compounds in Organic Synthesis*; Gordon & Breach: New York, 1984.
- (12) Dubowchik, G. M.; Padilla, L.; Edinger, K.; Firestone, R. A. Amines That Transport Protons across Bilayer Membranes: Synthesis, Lysosomal Neutralization, and Two-Phase pK_a Values by NMR. *J. Org. Chem.* **1996**, *61*, 4676–4684.
- (13) (a) McIntyre, D. K. Ph.D. Thesis, California Institute of Technology, Pasadena, CA, 1982. (b) Sarker, H.; Greer, M. L.; Blackstock, S. C. Synthesis, Structure, and Conformational Dynamics of Bridgehead-Substituted Nitrosamines. Di-1-adamantyl nitrosamine and Di-1-norbornyl nitrosamine. *J. Org. Chem.* **1996**, *61*, 3177–3182.
- (14) Maeker, H.; Levy, S. Normal Lymphocyte Development but Delayed Humoral Immune Response in CD81-Deficient Mice. *J. Exp. Med.* **1997**, *185*, 1505–1510.
- (15) Todd, S.; Lipps, S.; Crisa, L.; Salomon, D.; Tsoukas, C. CD81 Expressed on Human Thymocytes Mediates Integrin Activation and Interleukin 2-Dependent Proliferation. *J. Exp. Med.* **1996**, *184*, 2055–2060.
- (16) Levy, S.; Todd, S.; Maeker, H. CD81 (TAPA-1): A Molecule Involved in Signal Transduction and Cell Adhesion in the Immune System. *Annu. Rev. Immunol.* **1998**, *16*, 89–109.
- (17) Oren, R.; Takahashi, S.; Doss, C.; Levy, R.; Levy, S. TAPA-1 the Target of the Antiproliferative Antibody Defines a New Family of Transmembrane Proteins. *Mol. Cell. Biol.* **1990**, *14*, 2207–2213.
- (18) Hemler, M. E. Specific Tetraspanin Functions. *J. Cell Biol.* **2001**, *155*, 1103–1107.
- (19) (a) Cha, J.; Brooke, J.; Ivey, K.; Eidels, L. Cell Surface Monkey CD9 Antigen Is a Coreceptor That Increases Diphtheria Toxin Sensitivity and Diphtheria Toxin Receptor Affinity. *J. Biol. Chem.* **2000**, *275*, 6901–6907. (b) Schmid, E.; Zurbriggen, A.; Gassen, U.; Rima, B.; ter Muelen, V.; Schneider-Schaulies, J. Antibodies to CD9, a Tetraspanin Transmembrane Protein, Inhibit Canine Distemper Virus-Induced Cell–Cell Fusion but Not Virus–Cell Fusion. *J. Virol.* **2000**, *74*, 7554–7561.
- (20) Rice, C. Is CD81 the Key to Hepatitis C Virus Entry? *Hepatology* **1999**, *29*, 990–992.

- (21) Crotta, S.; Stilla, A.; Wack, A.; D'Andrea, A.; Nuti, S.; D'Oro, U.; Mosca, M.; Filliponi, F.; Brunetto, R. M.; Bonino, F.; Abrignani, S.; Valiante, N. M. Inhibition of Natural Killer Cells through Engagement of CD81 by the Major Hepatitis C Virus Envelope Protein. *J. Exp. Med.* **2002**, *195*, 35–41.
- (22) Kelic, S.; Levy, S.; Suarez, C.; Weinstein, D. E. CD81 Regulates Neuron-Induced Astrocyte Cell-Cycle Exit. *Mol. Cell. Neurosci.* **2001**, *17*, 551–560.
- (23) Holland, E. C. Progenitor Cells and Glioma Formation. *Curr. Opin. Neurol.* **2001**, *14*, 683–687.
- (24) Hirsch, T.; Decausin, D.; Susin, A.; Marchetti, P.; Larochette, M.; Resche-Rigon, M.; Kroemer, G. PK11195, a Ligand of the Mitochondrial Benzodiazepine Receptors, Facilitates the Induction of Apoptosis and Reverses Bcl-2-Mediated Cytoprotection. *Exp. Cell Res.* **1998**, *241*, 426–434.
- (25) Yoshida, D.; Cornell-Bell, A.; Piepmeyer, J. M. Selective Antimitotic Effects of Estramustine Correlate with Its Antimicrotubule Properties on Glioblastoma and Astrocytes. *Neurosurgery* **1994**, *34*, 683–687.
- (26) (a) Higginbottom, A. Identification of amino acid residues in CD81 critical for interaction with hepatitis C virus envelope glycoprotein E2. *J. Virol.* **2000**, *74*, 3642–3649. (b) Meola, A. Binding of hepatitis C virus E2 glycoprotein to CD81 does not correlate with species permissiveness to infection. *J. Virol.* **2000**, *74*, 5933–5938.
- (27) (a) Eckert, D. M.; Kim, P. S. Mechanisms of Viral Membrane Fusion and Its Inhibition. *Annu. Rev. Biochem.* **2001**, *70*, 777–810. (b) Debnath, A. K.; Radigan, L.; Jiang, S. Structure-Based Identification of Small Molecule Antiviral Compounds Targeted to the gp41 Core Structure of the Human Immunodeficiency Virus Type 1. *J. Med. Chem.* **1999**, *42*, 3203–3209.
- (28) Suzuki, T.; Yamaya, M.; Sekizawa, K.; Hosoda, M.; Yamada, N.; Ishizuka, S.; Nakayama, K.; Yanai, M.; Numazaki, Y.; Sasaki, H. Bafilomycin A(1) Inhibits Rhinovirus Infection in Human Airway Epithelium: Effects on Endosome and ICAM-1. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2001**, *280*, 1115–1127.
- (29) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. Safe and Convenient Procedure for Solvent Purification. *Organometallics* **1996**, *15*, 1518–1520.
- (30) Glaser, R.; Geresh, S. Steric and Electronic Factors in the Asymmetric Homogeneous Hydrogenation of Z- α -Acylaminocinnamic Acids and Esters Catalyzed by Rhodium(I) Complexes of DIOP. *Tetrahedron* **1979**, *35*, 2381–2387.
- (31) *InsightII*, version 97, and *Discover*, version 2.98; Molecular Simulations, Inc.: San Diego, CA, 1997.
- (32) Geisert, E., Jr.; Yang, L.; Irwin, M. H. Astrocyte Growth, Reactivity, and the Target of the Antiproliferative Antibody, TAPA. *J. Neurosci.* **1996**, *16*, 5478–5487.
- (33) McCarthy, K. D.; de Veillis, J. Alpha-Adrenergic Receptor Modulation of Beta-Adrenergic, Adenosine and Prostaglandin E1 Increased Adenosine 3'; 5'-Cyclic Monophosphate Levels in Primary Cultures of Glia. *J. Cycl. Nucleotide Res.* **1978**, *4*, 15–26.
- (34) Kueng, W.; Sibling, E.; Eppenberger, U. Quantification of Cells Cultured on 96-Well Plates. *Anal. Biochem.* **1989**, *182*, 16–19.

JM020326D